cultural medium. We used SR151716 (SR1) 1 mcM (Sanofi) as antagonist of CB1 receptors, SR 141716A (SR2) 1 mcM as antagonist of CB2 receptors. The main parameters of spontaneous neural and calcium activity as well as the viability of cells were investigated. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM (Invitrogen) and a confocal laser scanning microscope (Zeiss LSM510, Germany). The viability of dissociated hippocampal cells was evaluated according to the percentage ratio between the number of dead cells stained by propidium iodide (Sigma, Germany) and the total number of cells stained by bisBenzimide (Invitrogen, USA) for 7 days after hypoxia. Study the expression of mRNA CB1 receptors was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy.

RESULTS

The effect of N-ADA on the neural network activity and cellular viability in primary hippocampal cultures during hypoxia were investigated. Our experiments showed that 10-minutes acute hypoxia caused the significantly decrease in cellular viability of primary hippocampal cultures (in 4.5 times, $p<0.01$) in the posthypoxic period. N-ADA application maintained the viability of cells at level appropriate normoxic conditions (no significant differences from sham). Blockade of CB1 receptors by SR1 eliminated the N-ADA neuroprotective effect. Electrophysiological and Ca^{2+}-imaging data demonstrated that hypoxia causes a catastrophic reduction of spontaneous bioelectrical and calcium activity in primary hippocampal cultures and changes in activity patterns. Cannabinoid receptors activation via N-ADA applications during hypoxia partially preserved the spontaneous bioelectrical and calcium activity of neural networks for 7 days after hypoxia modeling. N-ADA neuroprotective effect associated with cannabinoid receptors type 1, as their blockade by using antagonist SR141716A (SR1) exterminate the neuroprotective effects of N-ADA. CB2 blocking by application of antagonist SR144528 (SR2) had no substantially effect. In this regard, we evaluated the level of CB1 receptors expression in hypoxic conditions. Our data revealed that mRNA CB1 is actively synthesized both neurons and glial cells. N-ADA was significantly ($p <0.05$, ANOVA) reduced the number of mRNA CB1 positive cells in primary hippocampal culture at normoxia. In the control group mRNA CB1 expression was detected in 37,7 ± 5,41% of cells and in 24,4 ± 7,26% of cells 48 hours after N-ADA (10 mM) application. Thus, it was shown that exogenous N-ADA application leads to changes the level of mRNA expression of cannabinoid receptor type 1. Hypoxia caused the increase of mRNA CB1 expression especially in astrocytes.

CONCLUSIONS

Therefore, it was shown that N-ADA has strong antihypoxic and neuroprotective properties. The protective N-ADA effect primarily implemented through CB1 receptors.

ACKNOLEGMENTS

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Growing Unidirectional Synaptic Architecture In Dissociated Neuronal Cultures Using Microfluidic Methods

Y. Pigareva [1], A. Gladkov [1,3], V. Kolpakov [1], E. Malishev [2], V. Kazantsev [1], I. Mukhina [1,3], A. Pimashkin [1]*

[1] Nizhny Novgorod Neuroscience Center, Institute of Biology and Biomedicine, Lobachevsky State University of Nizhny Novgorod, 23 Gagarina ave., Nizhny Novgorod, Russia, 603950

[2] Saint-Petersburg Academic University - Nanotechnology Research and Education Centre of the RAS, Saint-Petersburg, Russia

[3] Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia

[*] Corresponding e-mail: risssum@gmail.com

Summary. In this study we developed microfluidic structure with two neuronal cultures grown in separated chambers and connected by microchannels for axon outgrowth. We estimated bursting activity transfer characteristics between chambers in relation toculture development and determine rate of axonal growth in chambers.

Key words. Microfluidic system, microchannels, microelectrode array.

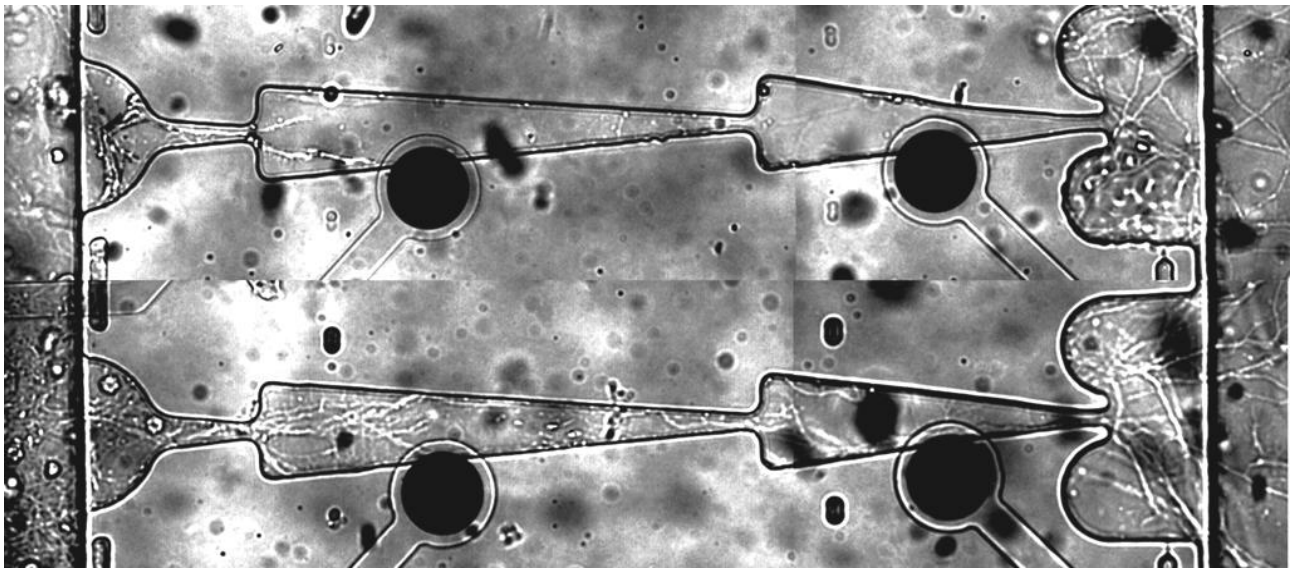

Fig. 1.Axons outgrowth in microchannels on day 2(a) and day 4(b)

INTRODUCTION

Microfluidics and microelectrode technology recently have been advanced in neuroengeneering in fundamental research of the brain and medical applications. Neuronal cells grown in microfluidic structures allow to isolate the cell populations in individual compartments connected by microchannels in which axons and dendrites can grow. The cultivation of neural networks on the microelectrode arrays using multichannel electrophysiology system makes possible to observe development and functional state of connections, their enhancement or suppression.

RESULTS

In this study we designed a microfluidic device combined with microelectrode arraywhich allows to grow two separate cultured neuronal networks with directed synaptic pathways inbetween. Microchannels' length was 400 µm and 600 µm.We found that 4 days was enough for axons to growthrough the whole channel from presynaptic chamber (chamber A) to postsynaptic chamber (chamber B) (Fig.1). The averagevelocity of axonal growth in microchannel was found to be13.8 µm per hour. Next we investigated spontaneous activity propagationthrough axonal pathways from one chamber to another during 30 days ofculture development. Specific design of the microchannels defined axon outgrowth. Percent of burst's transmitting in intended direction was changed depending on number of burst evoked in neuronal culture and increasedduring maturation (Fig. 2, c). Percent of the burst's propagation in opposite direction decreasedduring development (Fig. 2, d) and the ratio of directed against backward propagation increased (Fig. 2, e). We suppose that with culture development strength of synaptic connection increased and the activity propagated primary inintended direction.

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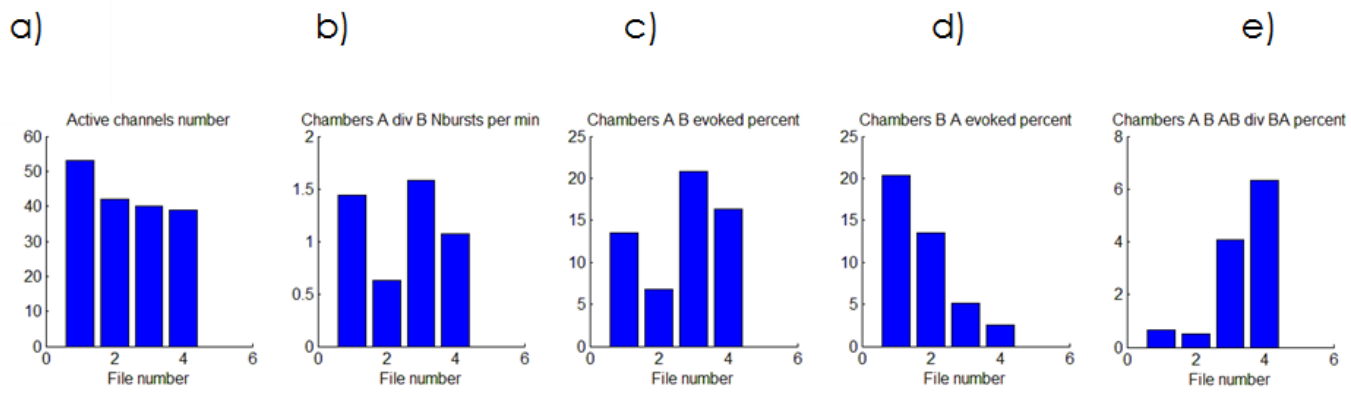

Fig. 2.Change of spontaneous signal propagation during culture development (15, 20, 25 , 30 days in vitro).The number of active electrodes (a), ratio of spontaneous bursting frequency recorded in two chambers (b), percent of bursts evoked from chamber A to chamber B (c), from chamber B to chamber A (d) and their relation (d).

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Microfluidics Applications In Fundamental And Medical Studies In Neuroscience

A. Pimashkin [1], A. Gladkov [1,3], V. Kolpakov [1], Y. Pigareva [1], E. Malishev [2], A. Bukatin [2], V. Kazantsev [1], I. Mukhina [1,3]*

[1] Nizhny Novgorod Neuroscience Center, Institute of Biology and Biomedicine, Lobachevsky State University of Nizhny Novgorod, 23 b. Gagarina ave., Nizhny Novgorod, Russia, 603950

[2] Saint-Petersburg Academic University - Nanotechnology Research and Education Centre of the RAS, Saint-Petersburg, Russia

[3] Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia

[*] Corresponding e-mail: pimashkin@neuro.nnov.ru

Summary. Today many fundamental questions in Neuroscience can be addressed with Microfluidics methods which provide unique approaches for cell patterning and control neural branch outgrowth. Such methods can be used to gwoe separate subpopulations of dissociated neurons connected with uni-directional connectivity. Combined with microelectrode arrays such approach can be used to simulate and study neurogenesis, learning and information coding in neural networks.

Key words. Neuronal cultures, microelectrode arrays, PDMS microchannels, synaptic plasticity.

INTRODUCTION

Today many fundamental questions in Neuroscience such as neuron synaptic coupling in various conditions, synaptic connectivity in the network, morphological structure and cell layers formation and many others require a development of new methods for cell manipulation and observation. Microfluidic chips containing chambers for cell plating can be easily fabricated and used for long-term imaging. and electrophysiological signal registration on MEA (microelectrode arrays).

RESULTS

In this study we designed a microfluidic device combined with microelectrode array which allows to grow two separated neuronal cultured networks with directed synaptic pathways inbetween into pre- and postsynaptic subpopulations of neurons. We investigated signal propagation through axonal pathways using different shapes of the microchannels in order to find optimal method to control axon outgrowth. After 10 DIV the axons coupled two cultures through 8 microchannels. We found that spontaneous bursts in presynaptic chamber evoked burst in postsynaptic chamber. Also we tested the direction of synaptic pathways in the microchannels by electrical stimuli applied to random electrodes in the pre- and postsynatic chamber. Then we designed PDMS chips with two-chambers and three-chambers to investigate different methods of synaptic connectivity modification between neuronal subpopulations. We used high frequency paired pulse stimulation to evoke potentiation of the synapses between neurons in separated chambers. Such direct approach can be used for study of synaptic plasticity on the network level. Also we used microfluidic device to study progenitor cells differentiation in presence of growth factors expressed by other neurons in order to find optimal conditions for neural tissue regeneration. One separate chamber was used to grow the cells dissociated from embryonic mice (E18) and other chamber for neurospheres (hippocampal progenitor cells E14). We found that progenitor cells differentiated in 7 days and formed synaptic connectivity with mature culture (E18) grown in opposite chamber. Such methods can be used to investigate stem cells and progenitor cell differentiation and functional integration in the brain. The results can be used in the study of neuroprostethics, neuroreabilitation.

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